REMARKS

Claims 19-24, 26-28, and 30-35 are pending.

This amendment is supported throughout the specification. No new matter is added by this amendment.

Rejections Under 35 USC §112

Claims 19-24, 26-28, and 30-35 have been rejected under 35 USC §112, first paragraph. Claims 19-24, 26-28 and claims 30-35 have been rejected under 35 USC 112, second paragraph. Claims 21 and 26, and new claims 31, 34 and 35 are stated to be indefinite.

Applicants traverse this rejection and point to the written support in the specification teaching what the contaminating levels of helper virus in a recombinant AAV preparation are after four rounds of cesium chloride centrifugation.

The specification teaches that, as measured by the assay described on page 35, lines 1-5, rAAV purified according to the invention, contains *no* detectable amounts of contaminating adenovirus. Given this description, Applicants submit that the meaning of this language is clear and definite.

The specification describes a method of detecting the amount of contaminating adenovirus in a rAAV preparation. See, page 35, lines 1-5, which summarize a method described in Fisher et al, J. Virol, 70:520-532 (1996), which is incorporated by reference in the present application and also in the priority documents. In addition, various other methods for detecting contaminating adenovirus, e.g., PCR analysis, have been known to those of skill in the art as of the priority date of this application. Thus, the specification provides sufficient teachings as to permit one of skill in the art to determine the amount of contaminating adenovirus in a rAAV preparation, regardless of the method by which the rAAV preparation is purified.

The inventors are the first to have found that it is the contamination of rAAV preparations with adenoviral helper virus which induces an immune response upon

delivery of rAAV delivery vectors which are so contaminated. Thus, the present inventors are the first to have described that it is the purification of rAAV away from adenoviral helper-virus that causes reduction or elimination of a cytotoxic immune response following rAAV delivery. See, e.g., Figs 5A-C, Example 5 and Example 7. Applicants have shown that four rounds of cesium chloride gradient centrifugation is a method for providing an rAAV preparation with a level of purity from adenoviral helper virus which eliminates cytotoxic immune response. However, as discussed above, four rounds of cesium chloride gradient centrifugation is not the only method by which adequate levels of contaminating adenoviral helper can be removed and the invention should not be limited to purification by only this number of steps of this particular purification method.

More particularly, given the teachings in the specification, one of skill in the art can readily determine both quantitatively and/or qualitatively the meaning of the phrase "at least as free of the contaminating adenoviral helper virus" as used in the present invention to describe the level of purity of the recombinant AAV of the invention.

Reconsideration and withdrawal of these rejections is requested.

Double -Patenting

The double-patenting rejection over issued US Patent 5,866,552 has been maintained. Provisional double-patenting rejections over co-pending US Application No. 09/757,673 and US application No. 09/237,064 have been raised.

Applicants agree to file a terminal disclaimer over the '552 patent. Applicants also agree to file terminal disclaimers with respect to each of the co-pending applications contingent upon the provisional nature of the rejection being removed prior to issuance of this application.

Rejection under 35 USC §103

Claims 19-24, 26-28, and 30-35 have been rejected under 35 USC §103(a) as being unpatentable over Podsakoff et al, US Patent 5,858,351, taken in view of Kashyap et al, J. Clin. Invest., 96:1612-1610 (September 1995). The examiner indicates that applicants have failed to provide evidence as to how to differentiate the impurities of an AAV preparation purified by one or two rounds of cesium chloride centrifugation from four rounds of purification.

Applicants traverse this rejection.

As previously noted, Podsakoff does not suggest the use of ApoE and Kashyap does not suggest the delivery of ApoE via a rAAV vector. Further, the combined documents fail to recognize the level of purity necessary for an rAAV to obtain the results provided by the present invention.

In the present specification, it is noted in Fisher et al, which is incorporated by reference for the purification method described therein, that:

> "Routinely, the purification scheme described above removed all detectable H5.CBALP [adenoviral] helper virus by the third round of buoyant density ultracentrifugation." Page 521, column 2, last sentence of paragraph beginning in column 1.

This teaching makes it clear that at least three rounds of CsCl centrifugation are required inadequate to remove helper virus from rAAV preparations. The present application describes the performance of yet an additional (i.e., fourth) round of CsCl centrifugation to ensure that any possible remaining contaminants have been removed.

Further, the present invention provides compositions which are 2 logs more pure of contaminating wild-type (wt) AAV than Podsakoff. Note, when purified as described in the present invention, rAAV preparations contain <1 infectious unit wt AAV per 10^9 genomes rAAV. See, page 35, lines 5-6 of the specification. In contrast, Podsakoff detects wt AAV contamination of approximately 1 in 10^7. See, col. 19, lines 16-17 of the '351 patent. One of skill in the art will readily understand the removal of wt AAV

reduces (or eliminates) the possibility of homologous recombination between the wtAAV and the rAAV of the invention.

Thus, the rAAV of the present invention are more pure than taught by Podsakoff. Again, it is noted that Kashyap fails to suggest anything about the use of rAAV vectors. Thus, the combination is deficient.

In addition, neither of the cited documents recognizes the importance of removing contaminating adenoviral helper from rAAV preparation to the level of purity required by the present invention. Notably, following Podsakoff's rAAV purification, he teaches heat-inactivation which will destroy the function of Ad. Thus, Podsakoff appears to be focused upon eliminating the function of the contaminating adenoviral helper, rather than removing the adenoviral helper itself. See, Podsakoff, col. 1, lines 52-55: "[A]denovirus vectors express viral proteins that may elicit an immune response which may decrease the life of the transduced cell.". Further, Podsakoff postulates that "the adult muscle cell may lack the receptor which recognizes adenovirus vectors, precluding efficient transduction of this cell type using such vectors." [Col. 1, lines 58-56]. Thus, Podsakoff's motivation is to use rAAV because he believes that there are insufficient adenoviral receptors in muscle cells. Podsakoff does not recognize that the mere presence of contaminating adenoviruses (even in the absence of the ability to express adenoviral proteins) may cause an immune response. Thus, Podsakoff does not suggest a solution which avoids even heat-inactivated adenoviral contaminants.

It is only the inventors who have recognized the significance of eliminating adenoviral contamination *and not just contamination by adenoviral function* which led to the present invention.

The combined teachings of the cited documents do not suggest the present invention.

Applicants request reconsideration and withdrawal of this rejection.

Applicants request that the examiner telephone applicants if doing so would expedite handling of this application.

Attached hereto is a "**Version With Markings to Show Changes Made**" (Appendix A) and a "**Clean Copy of Pending Claims Without Markings**" (Appendix B).

The Director of the U. S. Patent and Trademark Office is hereby authorized to charge any deficiency in any fees due with the filing of this paper or credit any overpayment in any fees paid on the filing, or during prosecution of this application to Deposit Account No. 08-3040.

Respectfully submitted,

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Appendix A
Version with Markings to Show Changes Made

21 (Five Times Amended). A composition comprising a recombinant adeno-associated virus (AAV) suspended in a biologically compatible carrier,

wherein said recombinant AAV comprises (a) a 5' AAV inverted terminal repeat[s] (ITR[s]), (b) nucleic acid sequences encoding human apolipoprotein E (ApoE) operably linked to regulatory sequences which direct its expression, and (c) a 3' AAV ITR[s], and

wherein the recombinant AAV is at least as free of contaminating adenoviral helper virus as is obtained by subjecting said recombinant AAV to four rounds of cesium chloride gradient centrifugation.

26(Twice Amended). A method of delivering apolipoprotein E (ApoE) to a mammal with atherosclerosis, said method comprising the step of

administering to the mammal a composition comprising a recombinant adeno-associated virus (AAV) suspended in a biologically compatible carrier,

wherein said recombinant AAV comprises (a) a 5' AAV inverted terminal repeat[s] (ITR[s]), (b) nucleic acid sequences encoding human apoliprotein E (ApoE) operably linked to regulatory sequences which direct expression thereof and (c) a 3' AAV ITR[s], wherein the recombinant AAV is at least as free of contaminating adenoviral helper virus as is obtained by subjecting said recombinant AAV to four rounds of cesium chloride gradient centrifugation

and wherein the ApoE in said composition is expressed in the mammal.

31(Amended). A method, said method comprising the step of

administering to the mammal a composition comprising a recombinant adeno-associated virus (AAV) suspended in a biologically compatible carrier intramuscularly,

wherein said recombinant AAV comprises (a)) a 5' AAV inverted terminal repeat[s] (ITR[s]), (b) nucleic acid sequences encoding human apoliprotein E (ApoE) operably linked to regulatory sequences which direct expression thereof and (c) a 3' AAV ITR[s],

wherein the rAAV is at least as free of contaminating adenoviral helper virus as is obtained by subjecting said recombinant AAV to four rounds of cesium chloride gradient centrifugation

and wherein the ApoE in said composition is expressed in the mammal.